

# MODERN INTERPRETATIONS OF SERUM TESTS\*

BY

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Since the advent of the new chemotherapy the treatment of venereal diseases has undergone a revolution which has not only affected the testing of sera from patients under treatment, but also the diagnosis of the diseases. It is therefore proposed to give an account of how we at the Whitechapel Clinic and the Venereal Disease Reference Laboratory are attempting to deal with this situation, and to indicate as clearly as possible the lines along which we are working. Clinicians may thus be enabled to understand our interpretations of serum reactions in the light of new developments.

The serology of these diseases had lagged behind, and this failure to keep pace was enhanced by the 1939-45 war. Since that time, however, much lost ground has been regained and progress is being made along the following four lines: (1) the standardization of reagents; (2) improvements in technique; (3) new tests; and (4) increasing use of the Venereal Disease Reference Laboratory.

## The Standardization of Reagents

The most notable work in the standardization of reagents was probably done by Richardson, as a result of which he published in the *Lancet* in 1941 a paper entitled "The Preservation of Liquid Complement Serum." Richardson's work did not receive the recognition it deserved. As a result of his investigation he produced a complement that for practical purposes was heat-stable. I have carried out experiments on this preserved complement by placing a bottle of this reagent in a 37° C. incubator and leaving it there for fourteen days. The complement was titrated before the heat was applied, and was then found to yield a titre of 1-80, and a similar reading was obtained after five days in the incubator at 37° C. After seven days the titre had dropped to 1-60, and after fourteen days to 1-10. Further experiments were conducted using the preserved complement in standard complement titrations. It was found that the loss of potency due to heat

incubation from contact with the other reagents used in the test was very little. Thus it is evident that for practical purposes this reagent is heat-stable. To those of us who remember the days when we were taught that complement must be kept cool until the last minute, and that allowance must be made for a drop in potency during the fixation period, the instability of this reagent was of great importance. It is now possible to calculate the doses of complement required in a test, and to have confidence that the titre will not drop appreciably during the test. Thus it is possible, without danger of upsetting the test, to make the test much more sensitive than was possible before Richardson's preserved complement was evolved.

With regard to the other reagents used in the test, only one other standardization is possible, that of the antigen. Attempts to make this standardization a reality have continued, and as a result improvements can be recorded.

## Improvements in Technique

There have been constant improvements in technique ever since the first Wassermann reaction was evolved. At first an immense amount of work was done, but this gradually lessened and techniques of the Wassermann reaction tended to remain static in this country during the ten-year period preceding the last war. Then fresh investigations began to be undertaken. In 1940, Richardson published a paper in the *British Journal of Venereal Diseases* entitled "The Specificity of the Bordet-Wassermann Reaction. Preliminary Note on an improved method." Since then the tempo has increased, particularly in America, and work at present proceeding at the Venereal Disease Reference Laboratory looks like yielding results. Here the method adopted has been the titration of antigens by the technique of optimal proportions. If our hopes are fulfilled we shall be able to guarantee that each batch of antigen produced is not only similar in sensitivity but very much more sensitive than any we have used heretofore. This would be a great gain, because

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we know from past experience that various batches of antigen do vary in potency, and as a result the sensitivity of the test also varies.

Many people express opinions in favour of the employment of a standard technique throughout the country. This seems inadvisable on two counts:

1. If a standard technique is employed by all venereal disease serologists there is likely to be little check on slight deviations from the standard. In the long run these slight deviations may result in a poor quality test. Moreover, it might be argued by some that since a standard technique is used comparative testing is unnecessary.

2. If all serologists are made to conform to one standard technique they may be deterred from attempting to improve on the "official technique."

Better than a standard technique would be a scheme of comparative testing between all laboratories concerned in performing Wassermann reactions. In fact it might reasonably be asked whether this was not the time to put such a scheme into practice. It would be easy to do so within the various regions of the National Health Scheme, and then later to adapt it to a national scale.

At the same time it would be well to standardize the reagents used in the tests and to issue from certain laboratories standard reagents guaranteed to work within limits and under reasonable conditions.

Another need is for a standard method of recording results. If any amount of serum be taken as the standard unit for recording results, and all positive reactions are recorded in terms of the amount of serum, one would then have an idea of the sensitivity of any particular technique. Thus, the positive results of any technique could be recorded as, "standard Serum Unit, Positive, serum diluted 1 in whatever dilution was calculated." This is not so difficult as it may appear; for example, in the Harrison-Wyler technique 0.022 ml. of serum is used, and if any positive result obtained by this technique at a definite dilution were adjusted by use of an appropriate factor this would give a serum dilution of positivity in terms of the standard amount of serum. Thus, if the standard amount of serum used in the recording of positive results were 0.1 ml. of serum the factor in this case would be 4.5. Mathematically this is a rough and ready method, but at least it would enable a clinician to compare in terms of positivity serum reactions results from any laboratory using any technique. If this method were not acceptable it would be possible to use a standard amount of serum, say 0.1 ml., in all techniques. This would involve altering the relative proportions of all the reagents used in each technique but would probably give similar results. As a small step towards this I am now reporting the Wassermann reaction, the Price precipitation reaction, and the complement fixation test for gonorrhoea in terms of a standard amount of serum, 0.11 ml.

#### New Tests

Many of the new tests which have been evolved are precipitation tests, but there is also the much publicized cardiolipin Wassermann reaction.

**Harrison-Wyler and Cardiolipin Tests.**—During the last few months my colleague Dr. Wilkinson and I have been testing sera in parallel using the standard Harrison-Wyler Wassermann technique and the cardiolipin Wassermann reaction. We have not been able to carry out the technique advocated by the originators of this reaction for the time factor precludes this, but we have adapted it to our own technique and titrated the antigen, obtained from America, by the optimal proportions method. At the time of writing this paper only a relatively small number of sera have been tested, namely 1,982, but an analysis of the results so far obtained may give a pointer to the value of this antigen. It is hoped to publish a full account of this work later.

Of the 1,982 sera tested by both methods, 1,902 (96 per cent.) were in complete agreement. The disagreements amounted to 80 (4 per cent.). These eighty disagreements included four cases of primary syphilis in which the cardiolipin Wassermann reaction was positive and the standard Wassermann reaction negative. There were also sixty-five sera from old treated syphilitics in which the cardiolipin Wassermann reaction was still positive and the standard test had become negative, and eleven sera which were possible false-positive reactions but are still under investigation. Of the total number of sera tested, thirty-one were from cases of primary syphilis, and it is of interest to note that of these sera in both tests thirteen were negative and fourteen were positive. Of the remaining four the cardiolipin Wassermann was positive and the routine Wassermann was negative. Parallel quantitative standard Wassermann reactions and cardiolipin tests also reveal that the cardiolipin antigen yields positive results in higher dilutions of serum. Thus, of forty-five sera tested in this way, cardiolipin yielded thirty-two stronger reactions, seven equal, and six weaker. When using doubling dilutions of serum, cardiolipin usually yields a positive result one dilution higher than the standard Wassermann. Thus it would appear that the cardiolipin antigen is more sensitive than the standard Wassermann antigen, and probably as specific. The number of sera tested is small, and therefore these opinions are not put forward as final. Some of the sera to be tested in the future will have to come from a group of patients in whom syphilis is not suspected. At the same time it may be presumed that the results obtained so far give an indication of what may be expected.

**Price's Precipitation Reaction.**—For several reasons it was decided at the Whitechapel Clinic to discard the Kahn test in favour of Price's precipitation reaction. First, the preparation of the antigen

is so simple that any reasonably well equipped laboratory is capable of undertaking it. Secondly, the test is evolved upon the principle of optimal proportions. Thirdly, it has now been in routine operation for at least three years, and as a confirmatory test I believe that it is more reliable than the Kahn test. Fourthly, the results are more easily read than the Kahn test. This test will be discussed later in relation to the Wassermann reaction.

#### Increasing Use of the Venereal Disease Reference Laboratory

The Venereal Disease Reference Laboratory has been in existence for many years. It exists for four main purposes :

1. The supply of standard reagents for serological testing.
2. For testing any sera which give anomalous results elsewhere. When clinicians want serum tested at this laboratory they should ask their own pathologists to split the specimen : they should then keep half themselves and send the other half to the Reference Laboratory. By this means results on the same specimen can be compared and a lot of unnecessary irritation avoided.
3. Research work, done mainly with a view to solving practical problems of the day and improving techniques. As time goes on the scope of these researches will be enlarged.
4. Confirmatory work on original research done elsewhere.

#### Complement Fixation Test for Gonorrhœa

After this brief survey it will now be well to consider the individual tests employed.

The first test to be considered is the complement fixation test for gonorrhœa, for this was the first of the serum tests to feel the impact of the revolution in the treatment of venereal diseases. Changes began to be noticeable before the war, but the full effect did not show itself until penicillin had been adopted as a routine in the treatment of gonorrhœa. A project to make a survey of the test during the war was frustrated by shortage of staff and incomplete information due to constant movement of patients to other areas. By the time such a survey became possible, penicillin had appeared in civilian clinics and quite soon after this it became obvious that the complement fixation test for gonorrhœa was not as useful as it had been. In due course an evaluation of the test was undertaken by my colleague, Dr. Curtis, and myself. In order to do this, all patients attending the Whitechapel Clinic suffering from gonorrhœa were subjected at first to twice-weekly tests, and later to weekly tests. This routine was started in April 1947, and it still continues. As a result it was possible to examine

the case records of 2,837 patients suffering from acute gonorrhœa. Dr. Curtis very kindly undertook this onerous task.

Of the total number of patients (2,837) under review, 1,430 had never had any signs or symptoms of genito-urinary disease before, and 1,407 had had a previous history of gonorrhœa.

The first thing that had to be determined was how soon after exposure to infection one might expect a positive complement fixation for gonorrhœa to be recorded, and in Fig. 1 a comparison is made with the results obtained in 1933.

It is evident on examination of it that one of two things must have happened : either the patient does not react as he did in the presulphonamide era, or

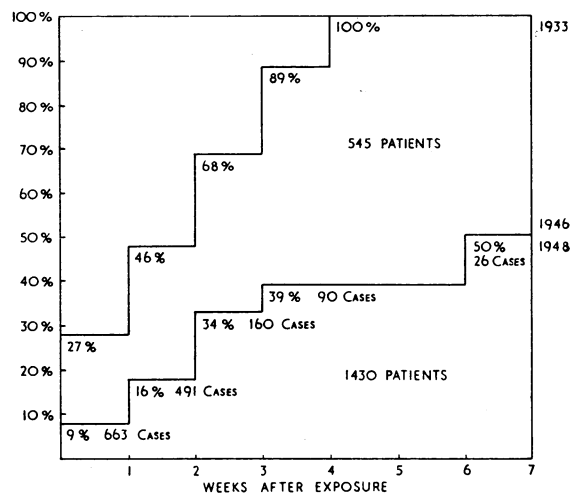


FIG. 1.—Complement fixation test for gonorrhœa : sero-positive time factor after exposure.

the complement fixation test for gonorrhœa is not as sensitive as it was. Possibly a combination of these factors is operating. The graph explains itself, but not the causes of these results. As far as the first point is concerned one can only indulge in speculation, but the second proposition is supported by some evidence. From experimental work which I have recently been doing I have reason to suspect that the antigen now produced is not so sensitive as in the old days. This may be due to the medium now employed. If our hopes are justified we shall be able to produce an antigen more sensitive than before and certainly much less anticomplementary.

The next step was to enquire as to what was the fate of the test during the course of treatment by penicillin, and for this purpose 959 patients who had two things in common were selected. First, they had not suffered from gonococcal complications or relapses, and secondly they all had had treatment

with 150,000 units of penicillin given as five injections each of 30,000 units in water intramuscularly two-hourly.

On examining the records of these patients we found that 652 (68 per cent.) failed to record a positive complement fixation test throughout the course of their illness; 185 (19.2 per cent.) were positive during the first week, that is, at the first testing, 61 (6.4 per cent.) became positive before the end of the first week; 24 more (2.5 per cent.) before the end of the second week, 7 before the end of the third week, 5 before the end of the fourth week, and 5 during the second month. In the remaining 19 patients the serum showed what we call "oscillation," that is, the serum tests recorded were negative, positive, negative, positive, or alternatively positive, negative, positive, negative.

From these figures it will be seen that the majority of patients (68 per cent.) failed to develop antibodies which could be demonstrated by the test, and presumably any reaction which might have taken place was prevented by the treatment. I have been unable to compare this figure with a parallel one obtained in sero-negative primary syphilis since in the latter case the second test is not done until fourteen weeks after the initial test, but comparative testing *might* yield similar results. Of the remainder the great majority developed antibodies before the end of the first week, probably before the treatment had any chance to take effect, and thereafter antibody production was on a diminishing scale.

"Oscillation" can possibly be explained on the theory that with any serum test a certain number of patients develop antibodies for a time to such an extent that they are constantly near the threshold of recognition, sometimes being over this threshold and sometimes just under. This process in gonorrhœa possibly goes on for a relatively short time until antibody production recedes and thereafter negative results are recorded.

When examining the case cards of the 185 patients who were positive at the initial test, the difficulty of obtaining information due to patients defaulting must be borne in mind.

For instance:

- 95 defaulted in less than a week and had only one test
- 8 defaulted after one week
- 11 defaulted after two weeks
- 8 defaulted after three weeks
- 8 defaulted after four weeks
- 11 defaulted after two months
- 4 defaulted after three months
- 4 defaulted after more than three months.

Therefore, when this group of patients came to be examined in order to find out how long the initial

positive test remained positive, one was left with a total of 36 patients out of the original 185. The serum became negative after the initial positive test and treatment with penicillin:

- in one week with 6 patients
- after two weeks with 4 patients
- after three weeks with 6 patients
- after two months with 5 patients
- after three months with 2 patients.

This left 13 patients whose sera showed "oscillation." From such a small number little information can be gleaned, but as might be expected there seems to be a steady decline in the production of antibodies.

Next a group of 102 patients was examined in whom the sera was negative at the first test but became positive at some time after treatment with penicillin.

Of these:

- 61 became positive before the end of the first week before treatment in all probability had had time to have any effect
- 24 became positive before the end of the second week
- 7 became positive before the end of the third week
- 5 became positive before the end of the fourth week
- 5 became positive before the end of the second month.

It will be seen, therefore, that the great majority of these patients became positive before treatment could have much effect, and as might be expected the number thereafter becoming positive decreased every week.

It is of interest to examine these 102 patients who were first negative and subsequently became positive. For instance:

- 66 were positive after one test only, and therefore a graph would be useless
- 17 became positive after two tests
- 9 became positive after three tests
- 5 became positive after four tests
- 15 became positive after eight tests.

If one attempted to draw graphs of these sero-positive tests, only the last three groups would be of much use.

It will thus be seen that whilst the majority of patients (652, 68 per cent.) remained negative throughout the disease, the rest (307, 32 per cent.) that had recorded positive results did so for only a very limited period, and *if one is to get any idea of antibody production, tests must be made at least at weekly intervals until such time as the complement fixation test for gonorrhœa remains negative.* At the same time there are a certain number of "oscillators" which must be reckoned with.

If one more examination of the figures is undertaken in order to demonstrate the production of antibodies after penicillin treatment, it can be shown that it was not until the second week that any patients who were originally negative and who were treated with penicillin subsequently became positive. Thus, taking 76 of these patients :

14 (19 per cent.) became positive during the second week  
30 (40 per cent.) became positive during the third week  
18 (23 per cent.) became positive during the fourth week  
5 (7 per cent.) became positive during the fifth week  
9 (11 per cent.) became positive during the sixth week.

From these results it would appear that in spite of the penicillin treatment antibody production was greatest in the second, third, and fourth weeks after penicillin had been given, and it would suggest that once the mechanisms of antibody production is activated, penicillin treatment does not prevent the initial stimulus, but prevents the continuation of this.

Finally, examinations of the figures were made from patients who suffered from complications. In the case of male patients these were taken from the period September 1945 to September 1948 and totalled 57. Of these, 36 were positive at the relevant times, 2 becoming positive after the complication had occurred and 19 remained negative throughout. As an example of complications in the female, all cases of salpingitis occurring between September 1946 and September 1948 were examined and it was found that, out of a total of 13, 12 were positive at the relevant time, and one which was recorded as undoubtedly gonococcal had a negative result. Surprisingly it would seem that at least two-thirds of gonococcal complications can be expected to record positive results.

From this analysis it will be seen that the complement fixation test for gonorrhoea is but a shadow of its former self, though it still merits, even in its present state, the attention of clinicians.

It is possible to come to the following conclusions :

1. In early acute gonorrhoea its use to the clinician is limited.
2. It is of some value in subacute or chronic gonorrhoea, particularly in the female, when smears or cultures may give little or no help initially.
3. Its use is limited in the management of gonorrhoea.
4. It is of definite value in the diagnosis of complications.

5. It is unwise to ignore a positive test which has been confirmed by retesting.

I now record all positive results in terms of the standard 0.11 ml. of serum used and positive results are quantitated as a routine.

As a final consideration it is well to remember that venereal disease serology is in a state of flux, and this applies with particular force to the complement fixation test for gonorrhoea.

### Wassermann and Price Precipitation Reactions

For the past four years serum tests for syphilis in the Whitechapel Clinic, when positive, have been recorded on a quantitative basis as a routine. The two tests employed are a modification of the Harrison-Wyler Wassermann technique and the Price precipitation reaction. The latter is a precipi-

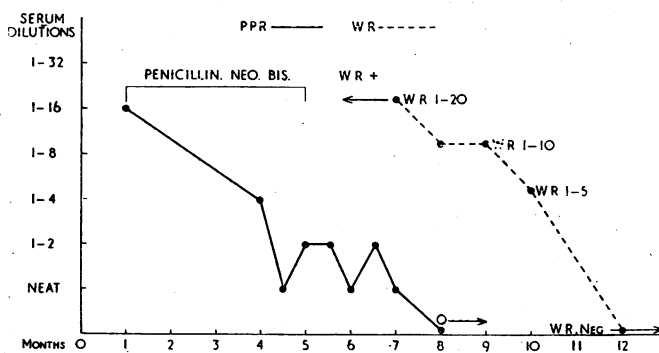


FIG. 2.— Serological test for syphilis: serology time chart.

tation test, details of which were published in the *Journal of Clinical Pathology* (Price, 1948). The work on this test had been going on and was completed two years previously, and the test was adopted in 1946 instead of the Kahn since it was considered that at that time it was at least as specific as the Kahn test and as sensitive as the Wassermann reaction. Moreover, the results were easy to read and the preparation of the antigen well within the scope of any reasonably equipped laboratory.

Since the time that the Price precipitation test (P.P.R.) was adopted the Wassermann has been made more sensitive, and the present position is that the Wassermann reaction is in most cases rather more sensitive than the P.P.R. and at least equal to the Kahn. At the present time, patients' sera when positive with the Wassermann and P.P.R. are recorded quantitatively in terms of the P.P.R. until such time as the Wassermann remains positive and the P.P.R. has become negative. From then on the Wassermann is done quantitatively until this, too,

becomes negative. This is shown in Fig. 2. At the outset both Wassermann and P.P.R. were positive. Penicillin was then given, followed by neoarsphenamine and bismuth, and both reactions remained positive for seven months. At the eighth month the stage was reached when the Wassermann was positive and the P.P.R. negative. Quantitative tests were then done on the Wassermann until finally this, too, became negative.

The reason for adopting this method of quantitative testing and recording of results is that whilst it gives the information that the clinician requires, at the same time it is economical in time and labour as far as the laboratory is concerned. It is suggested that for the convenience of the clinician all patients who record positive serum tests should have a graph plotted and that this should be kept at the beginning of the case card.

As a result of this quantitative testing we have adopted various terms to explain the different phases of the titre curve obtained. For instance, such terms as "falling titre" and "rising titre" explain themselves. The oscillating phase may be defined as that part of the curve recording positive and negative reactions in a fairly regular sequence. Finally the curve becomes stabilized, and thereafter usually registers negative results. Occasionally one gets an oscillating phase in the positive zone of the curve.

Fig. 3 illustrates an oscillating phase. At the beginning the Wassermann oscillates. Then at the sixth month both the Wassermann and the P.P.R. oscillate, both reactions becoming negative at the tenth month and remaining negative thereafter. It will be noted that the serum dilutions recording positive reactions are of a low order. There is another phenomenon which has been termed a "flutter." In the latter instance a patient's serum

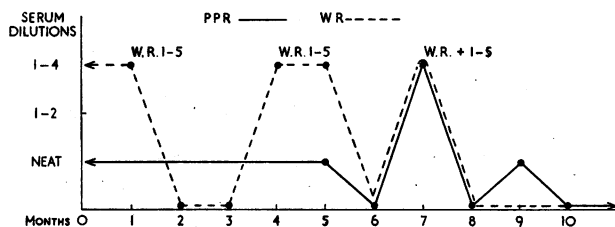


FIG. 3.—Serological test for syphilis: "oscillating" phase.

reactions have been negative for some time, and then for three or four consecutive reactions positive results at a low serum dilution are recorded, which slowly and quietly return to, and remain, negative. This phase usually occurs with the sera of patients who have had adequate treatment and are under

observation. This is illustrated in Fig. 4, which shows the results in a patient who had been under observation for six months with regular blood testing. At the third month shown on the curve the P.P.R. was positive using neat serum whilst the Wassermann remained negative. Both tests were

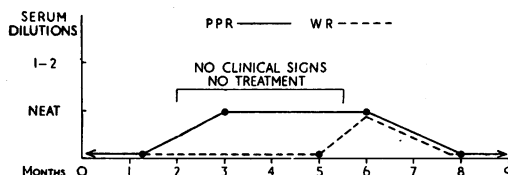


FIG. 4.—Serological test for syphilis: "flutter" phase.

positive at the sixth month, only to become negative at the eighth. Compared with an oscillating phase, which consists of a series of peaks, the flutter has a steady flat top.

**Increase in Wassermann Sensitivity.**—Having explained the terms that will be used in the following analysis of the results of parallel serum testing between the Wassermann and P.P.R., an attempt will now be made to show that the sensitivity of the Wassermann reaction has been increased. Technical details are omitted, since these will be of little interest to clinicians. Two groups of unselected sera were tested in parallel using the Wassermann and P.P.R. The first group of 4,170 were tested by the Harrison-Wyler technique and the P.P.R. between October 1947 and February 1948. The second group of 4,106 unselected sera were tested by an amended Wassermann technique and the P.P.R. between June and September 1948.

Table I is an analysis of the first group, of 4,170 sera. The two tests agreed in 4,059 (97.3 per cent.) instances and gave 112 (2.7 per cent.) discordant results, involving 87 patients. The number of patients involved in these discordant results has been analysed rather than the total number of serum reactions, in an attempt to give a truer picture of the position as far as the clinician is concerned.

The great majority of these discordant serum reactions occurred after the patients had been treated and the titres of the serum reactions were falling. Of these, the Wassermann was positive in 18 with the P.P.R. negative, and the P.P.R. was positive in 37 with the Wassermann negative. The oscillators were almost equal, as were the rising titres, whilst false positives were actually equal. If, therefore, one takes the falling titres as indicating the amount of reagin in the serum detected by any test, the results

of Table I tend to show that the P.P.R. at this time was rather more sensitive than the Harrison-Wyler Wassermann reaction.

From February 1948 until June 1948 the sensitivity of the Wassermann was increased, mainly by a stricter method of reading the complement titration. This was rendered possible by using Richardson's

TABLE I  
ANALYSIS OF GROUP OF SERA TESTED  
BY THE HARRISON-WYLER WASSERMANN TECHNIQUE  
AND THE P.P.R.

	No.	%
Total sera tested in parallel by Harrison-Wyler W.R. technique and the P.P.R. . . . .	4,170	
Agreements . . . . .	4,058	97.3
Disagreements . . . . .	112	2.7
Patients whose sera gave discordant results . . . . .	87	

Serum reactions	Rising titre	Falling titre	Oscillators	False positives	Total patients involved
W.R. pos. P.P.R. neg.	7	18	7	1	33
W.R. neg. P.P.R. pos.	7	37	9	1	54

preserved guinea-pig serum which, as I have already stated, is for practical purposes heat-stable, and one was, therefore, able to use very much less complement in the test without danger.

After much experimental work an amended Wassermann technique was adopted in June 1948. In order to show the increased sensitivity of this Wassermann reaction the results of all sera tested between that date and September 1948 have been examined.

The total number of sera tested in parallel using this amended technique of the Wassermann and P.P.R. was 4,106, a number approximating to that of the first group, 4,170. The number of disagreements in this series was 105 (2.6 per cent.) representing the sera of 52 patients, and these disagreements have been examined on the same principle as those of the previous series (Table II).

Of the falling titres where the Wassermann was positive and the P.P.R. negative there were 33, and in similar sera in which the P.P.R. was positive and the Wassermann negative there were only 5. Rising titres registered 3 with the Wassermann positive and P.P.R. negative, and none with a positive P.P.R. and negative Wassermann. Oscillators were 3 positive Wassermann and negative P.P.R. and 2

positive P.P.R. and negative Wassermann. Finally, the false positives with positive Wassermann and negative P.P.R. of which there were 3, and a further 3 who defaulted after one test and were therefore unclassifiable. On the other hand, with a positive P.P.R. and negative Wassermann there were no false positives.

Thus if one judges the relative merits of the amended Wassermann technique and the P.P.R. in a similar manner as one did the original Wassermann technique and the P.P.R. the conclusion is that the amended Wassermann technique is considerably more sensitive than the P.P.R.

The question that remains is, can the Wassermann reaction be further stepped up in sensitivity without producing more false positives? It may be possible by adjustment of the antigen, but since parallel tests have only just begun there is so far no useful information. However that may be, the position now is that the Wassermann is the most sensitive test and should be the test of choice with "antenatal bloods" and suspected early cases of syphilis. In the case of treated syphilis, whilst the P.P.R. usually becomes negative first, it is uncommon for it to

TABLE II  
ANALYSIS OF GROUP OF SERA TESTED BY THE  
AMENDED HARRISON-WYLER TECHNIQUE  
AND THE P.P.R.

	No.	%
Total sera tested in parallel by amended Harrison-Wyler W.R. technique and P.P.R. . . . .	4,106	
Agreements . . . . .	4,001	97.4
Disagreements . . . . .	105	2.6
Patients whose sera gave discordant results . . . . .	52	

Serum reactions	Rising titre	Falling titre	Oscillators	False positives	Total patients involved
W.R. pos. P.P.R. neg.	3	33	3	3* 3	45
W.R. neg. P.P.R. pos.	0	5	2	0	7

\* Serum reactions on these three patients were unable to be classified since the patients defaulted before assessment could be made.

revert to positive. Nevertheless it is wise to follow the Wassermann reaction until it, too, becomes negative.

**Biological False Positives.**—A definition of biological false positive reactions is difficult, and the number of diseases said to be the cause of such serum reactions is legion. One can assume that

the repeatedly positive blood test given by the serum of a patient who fails to yield any evidence of the disease is a biological false positive. On serial testing the blood test remains positive for a relatively short time, but gradually its strength fades until it becomes negative in the absence of any treatment. Taking this as the standard, out of the 8,276 Wassermann reactions recorded in the two groups already discussed there were 7 (0.085 per cent.) and 1 (0.01 per cent.) P.P.R. At the Venereal Disease Reference Laboratory the results of 1,036 sera sent in for comparative testing have been analysed, and it has been found that on the history submitted 12 (1.1 per cent.) could be classed as biological false positives.

As in this country we are not afflicted with tropical diseases, this problem of the biological false positive is not so acute as elsewhere. However, an investigation into the problem is now under way.

**Serological and Clinical Relapses.**—My colleague Dr. Nicol has given me the numbers of all clinical relapses occurring in female patients at the White-chapel Clinic from October 1946 until December 1948. These totalled 6, and 5 of them were preceded by a serological relapse. Fig. 5 shows the serology of a typical case. It reminds us that a rising titre in the serology after a period of negativity should be regarded with utmost suspicion, particularly in early syphilis.

On the other hand treated sero-relapses can be shown to respond to treatment without the super-vention of clinical manifestations. Fig. 6 illustrates this phenomenon. Here is a patient who

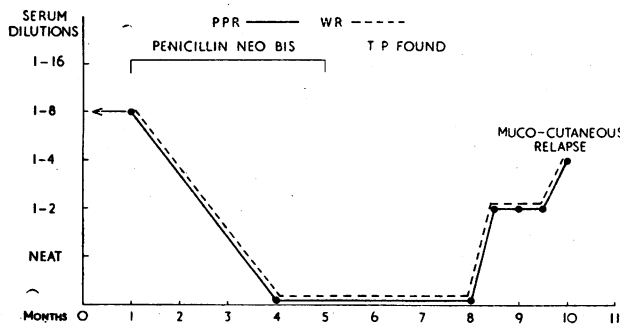


FIG. 5.—Serological test for syphilis: serum relapse.

suffered from two sero-relapses, the first of which responded partially to treatment.

Before leaving the subject of serum tests for syphilis it should be mentioned that high titred positive sera are as a rule associated with gummata, and, as might be expected, the contrary is often true of latent congenital syphilis.

### Concluding Remarks

From the foregoing remarks it will be seen that the interpretation of any serological test depends on the recognition of its capabilities, and since the time factor is all-important an accurate knowledge of the clinical history is essential. However, it will be seen that in the modern management of syphilis in

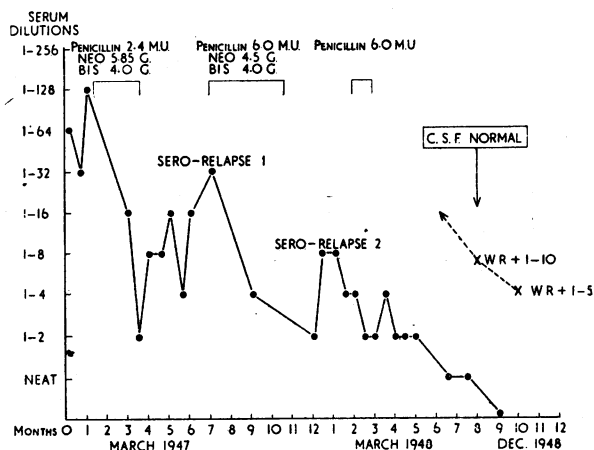


FIG. 6.—Serological test for syphilis: serum relapses.

particular a series of positive tests plotted as a curve is useful as a visual aid in assessing the serological progress of the case.

It should be remembered that, whilst there is no proof that the titre of any positive serum test indicates the reaction of the body against the disease, the possibility that it does so remains.

I certainly find it hard to believe that a positive serum test with a high titre indicates a severe infection. In fact such a result may augur a good prognosis, although exact information on this point is lacking.

If standard reporting of serological results on an agreed system were adopted, it is possible that the clinician would be in a better position to understand pathological reports, and at worst he would be able to evaluate the relative sensitivity of various techniques of serum tests for gonorrhoea and syphilis. Reform is desirable, but unless this Society in

particular and clinicians in general agitate much more in the future than they have in the past, little will be done.

Serologists should be given a brief history of each patient from whom a serum comes for testing. The shorter this statement the better, provided it contains the relevant facts such as the diagnosis, treatment



(if any), present state of the patient, and the results of the pathological tests (if any). This enables the serologist to check his work. Without this information bad work is apt to follow, irritation is caused, and no accurate interpretation of results can be given.

During the war we lost six years during which very little research work on serum tests was undertaken. Since the war we have been attempting to make this up, but the whole subject is in a state of

flux and will not settle down for some years. Therefore I trust that clinicians will deal gently with venereal disease serologists, who are constantly trying to improve their reagents and technique.

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## DISCUSSION ON MODERN INTERPRETATIONS OF SERUM TESTS

COL. L. W. HARRISON was very pleased at Dr. Price's reference to Dr. Richardson's method of preserving complement in the liquid state. As Dr. Price had said, that was a discovery which had received too little notice, and there was a danger that it might be forgotten and later be re-discovered in some other country, which would get the credit. This preserved complement was one of the useful discoveries which our dire need in the late war had stimulated. When Dr. Richardson, who was then in charge of the Ministry of Health (now the M.R.C.) Venereal Disease Reference Laboratory, had asked at the beginning of the war about the most useful lines of work for the laboratory, he (the speaker) had suggested the manufacture of reagents for serum tests, and the outcome was, *inter alia*, this preserved complement. At the beginning of the war he had made enquiries of the approved laboratories to discover how much need there was for such a product, and he had been surprised at the number of laboratories which were using very indifferent complement, from undersized guinea-pigs, from guinea-pigs which had been used for tuberculosis tests of milk, of indifferent titre, etc. Most used a single pig, and few as many as three or four animals. With complement serum obtained by bleeding for the batch of tests to be done on a given day, one had to be careful to keep it refrigerated almost up to the moment of its use in the test. On the other hand, the serum preserved according to Richardson's method could, as Dr. Price had said, be left lying about at room or even higher temperatures; it could be used to the last drop in the vial, and it could be made up in batches each from a pool of not less than a hundred male guinea-pigs of not less than 600 g. weight, each individual serum of the pool being tested for suitability before inclusion. He considered that this preserved complement serum was a most important contribution to standardization of the Wassermann test as, like the antigenic extract, it could be supplied from a central laboratory.

Dr. Price had expressed the hope—and he trusted that his hope would be realized—that with the use of complement preserved by the Richardson method one could go

closer to the borderline of safety and so make the Wassermann test more sensitive. That would be valuable. He remembered that in the early days of the "606" era he had constantly tried to make the test as sensitive as possible so that there should be as little danger as possible of their living in a fool's paradise. The result had been what was known as "No. 1 Method, M.R.C." It used three hæmolytic doses. As far as he could remember, "No. 3 Method, M.R.C." elaborated by Fildes and McIntosh, used two doses. He himself had thought that too little, but wondered how little Dr. Price now thought could safely be used.

He saw no harm in standardization of testing so long as the people concerned were trained personally; he believed, from experience, that very few serologists could practise an author's method exactly, if they relied only on his description. As evidence of this he would cite the case of what was now known as the Harrison-Wyler Method. When "No. 1 Method, M.R.C." had come through the ordeal of two international comparisons so creditably that it could confidently be recommended to the approved laboratories in this country, he had asked Dr. Wyler, who was then in charge of the Venereal Disease Reference Laboratory and had practised the method, to write a description of it in such detail that any intelligent serologist could copy it exactly. The result had been the Medical Research Council's Special Report No. 129, describing the Harrison-Wyler method, but although the description was so detailed that one could hardly imagine anyone deviating from it, of the many approved pathologists in the country who had said they practised the method, only those who had been taught it personally gave results equal to those obtained by Dr. Wyler with identical sera.

He agreed about the standard recording of results. That was something which the League of Nations Health Organization had tried to bring about. They had recommended, and the Ministry of Health had backed the recommendation, that results should be reported as positive, doubtful, or negative. The